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Pathogenicity of three species of entomopathogenic nematodes to some major stored-product insect pests

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Abstract

Entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) are commonly used biological control agents of insects in cryptic habitats, but their potential for suppressing stored-product insects in these habitats has not been explored previously. Here, we provide data from the first step in a program to evaluate entomopathogenic nematodes in the genus *Steinernema* as biological control agents of stored-product pests by determining their pathogenicity to some of the major stored-product pest species. When evaluated against larvae, pupae and adults of six pest species (*Plodia interpunctella*, *Ephestia kuehniella*, *Oryzaephilus surinamensis, Tenebrio molitor, Tribolium castaneum*, and *Trogoderma variabile*), and the adults of two additional pest species (*Sitophilus oryzae* and *Rhyzopertha dominica*), *Steinernema riobrave* was either the most pathogenic or of similar pathogenicity compared to *S. carpocapsae* and *S. feltiae*. A dose of 10 infective juveniles of *S. riobrave* caused 80% or higher mortality against larvae of *P. interpunctella*, *E. kuehniella*, *T. castaneum*, and *O. surinamensis*, pupae of *T. castaneum* and *T. molitor*, and adults of *T. molitor* and the two moth species. All stages of *Trogoderma variabile* exhibited 70% or higher mortality. Adults of *S. oryzae* and *R. dominica* exhibited low susceptibility with 15% and 35% mortality, respectively. On the basis of these results, *S. riobrave* was selected for further evaluation under more field-like conditions.

Keywords: *Steinernema* spp; Stored-product pests; Biological control; Entomopathogenic nematodes

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1. Introduction

Stored-product insects can have a large economic impact on stored bulk grain and processed commodities (Hagstrum and Flinn, 1995). These insects can survive on small amounts of food that accumulate in inaccessible places, such as cracks and crevices, under perforated floors, and inside machinery, and may move from these refugia into packaged and bulk-stored products (Campbell et al., 2004). The availability, effectiveness, or desirability of chemical pesticides that target insects in these cryptic locations is declining due to changes in government regulation (e.g., Food Quality Protection Act (FQPA), Montreal Protocol), development of resistance (Subramanyam and Hagstrum, 1995), and growing concern about chemical residues, worker safety, and shifting consumer demands that favor the adoption of more environmentally favorable management tools for stored-product pests.

Biological control may be an effective strategy for stored-product pest management in inaccessible locations, because some natural enemies can actively seek out pests in these hidden habitats or may be applied in a manner similar to chemical pesticides. However, most of the previous work on biological control of stored-product pests has focused on bulk-grain situations. For example, parasitic wasps (e.g. *Theocolax elegans* (Westwood) and *Anisopteromalus calandrae* (Howard)) have been shown to suppress pest populations effectively in bulk storage (Schöller and Flinn, 2000). Pathogens such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Nosema* spp., and *Mattesia* spp. have also been investigated, although there has been only limited field testing (Brower et al., 1995). The bacterium *Bacillus thuringiensis* (Berliner) (*Bt*) has been approved as a grain protectant in the United States (Brower et al., 1995), and is commercially available for the control of Indian meal moth larvae. Effective control using *Bt* has been reported against lepidopteran larvae attacking stored products, but resistance has also been reported (McGaughey and Beeman, 1988). Another pathogen that has been tested and is commercially available for the control of stored-product moths on dried fruits and nuts, or as a treatment in cracks and crevices, is a granulosis virus (Vail, 1991).

Entomopathogenic nematodes are lethal endoparasites of insects (Gaugler and Kaya, 1990; Gaugler, 2002). They enter their host through natural body openings, penetrate into the hemocoel, and release mutualistic bacteria that kill the host within 24–48 h and make the environment inside the insect suitable for nematode development. They have one free-living stage, the infective juvenile (IJ), which leaves a depleted host and actively finds and penetrates a new host. The other nematode life stages occur inside the insect where they feed on the bacteria and the host.

Entomopathogenic nematodes have been most widely used as biological control agents in soil environments (Kaya and Gaugler, 1993). However, entomopathogenic nematodes have many characteristics that make them potentially good biological control agents for stored-product pests. They have low toxicity to vertebrates (Bathon, 1996; Boemare et al., 1996; Kaya and Gaugler, 1993), they are exempt from registration in the United States by the EPA (Kaya and Gaugler, 1993), many species are commercially available (Grewal, 2002), they can be applied with conventional pesticide equipment (Hayes et al., 1999), they can tolerate many types of pesticides (Koppenhofer et al., 2000; Nishimatsu and Jackson, 1998), many species have a wide host range (Capinera and Epsky, 1992; Gaugler et al., 1997), and they have the ability to seek their host actively (Campbell and Lewis, 2002).

Entomopathogenic nematodes exhibit different search strategies to increase the probability of finding a host. Foraging strategies used by steinernematids vary from an ambush strategy to cruise foraging, with many intermediate types (Campbell and Gaugler, 1993, 1997; Campbell and Kaya, 2002; Grewal et al., 1994; Lewis et al., 1992, 1993). Cruisers move actively in search of hosts while ambushers exhibit more of a "sit and wait" strategy. Adoption of a foraging strategy has implications for other aspects of parasite ecology, behavior, physiology, and anatomy and thus influences how parasites interact with hosts (Campbell and Lewis, 2002). For this study we selected ambush to intermediate foraging species because of their environmental tolerance, small to medium size, and commercial availability.

Entomopathogenic nematodes have proven to be effective against a wide variety of insects in different environments. They have been tested in many systems (e.g., orchards, turfgrass, row crops) (Begley, 1990; Klein, 1990; Peters, 1996) and against urban pests such as cockroaches (Koehler et al., 1992; Zervos and Webster, 1989). These nematodes have been used to control members of some of the major pest families encountered in storage commodities, e.g., Pyralidae (Shannag and Capinera, 2000) and Curculionidae (Duncan and McCoy, 1996; Shapiro and McCoy, 2000). In an early survey, the susceptibility of some stored-product insects such as *Ephestia kuehniella* Zeller and *Tenebrio molitor* L. to a single high dose of nematodes was demonstrated (Morris, 1985). Heterorhabditid spp. efficacy against *P. interpunctella* (Hübner) under laboratory conditions has also been investigated (Mbata and Shapiro-Ilan, 2005).

Here, we compare three species of entomopathogenic nematodes in terms of their pathogenicity against multiple life stages of some of the major pest species of stored products. Multiple pest species were tested because, typically, stored-product pest management deals with a suite of pest species at the same location. The objective was to determine the most pathogenic nematode species against a wide range of pests under conditions for optimal infection. The selected species will be tested further under more realistic field conditions to assess their potential as biological control agents.

2. Materials and methods

2.1. Insects

Eight economically important insect species representing a diverse range of stored-product pests were selected as hosts. Six species of Coleoptera were chosen, including two internal feeders whose larval and pupal stages occur inside the kernel (the rice weevil [Curculionidae: *Sitophilus oryzae* (L.)] and the lesser grain borer [Bostrichidae: *Rhyzopertha dominica* (F.)]) and four secondary pests (the red flour beetle [Tenebrionidae: *Tribolium castaneum* (Herbst)], the sawtoothed grain beetle [Cucujidae: *Oryzaephilus surinamensis* (L.)], the warehouse beetle [Dermestidae: *Trogoderma variabile* Ballion], and the yellow mealworm [Tenebrionidae: *T. molitor*]). Two species of Lepidoptera (Pyralidae), the Indian meal moth [*P. interpunctella*] and the Mediterranean flour moth [*E. kuehniella*], were also tested. All species were obtained from colonies maintained at the Biological Research Unit, Grain Marketing and Production Research Center, USDA ARS in Manhattan, KS, except for *T. molitor*, which was obtained from Timberline (Marion, IL, USA).

2.2. Entomopathogenic nematodes

The assays were conducted using three species of entomopathogenic nematodes with different host search strategies: *Steinernema carpocapsae* (Weiser) utilizes an ambush strategy, whereas *Steinernema feltiae* Filipjev and *Steinernema riobrave* Cabanillas, Poinar, and Raulston exhibit an intermediate strategy. Nematodes were originally obtained from Harry K. Kaya at the University of California, Davis. They were reared in *Galleria mellonella* L. or *T. molitor* following the techniques described in Kaya and Stock (1997). Different batches of IJs were used for infections in each experimental block and only IJs less than two-weeks old were selected.

2.3. Insect susceptibility assays

Nematode pathogenicity was tested against last instar larvae, pupae, and adults of all insect species, except the internal feeders where only the free-living adult stage was tested. For each insect species and stage, individuals were exposed to either 0, 1, 5, 10, 20, 50, 100, or 200 IJs. Insects were exposed to nematodes individually in plastic micro-centrifuge tubes (1.5 ml) with a small hole in the lid (approximately 0.6 mm diameter) to allow air exchange. Each tube contained a small amount of food material (approximately 0.01 g) at the bottom and a 3.5×1.5 mm piece of grade 360 filter paper (Baxter Inc, McGaw Park, IL). The type of food in the tube depended on the insect's diet: lepidopteran species, *T. castaneum*, and *T. molitor* had flour; *O. surinamensis* and *T. variabile* had rolled oats; *R. dominica* and *S. oryzae* had cracked wheat. The assigned nematode dose was added in 50 µl of water to the filter paper and then the individual insect was added to the tube. Tubes were held at 90% relative humidity (r.h.) and 25 °C. Twenty replicates (two blocks of ten tubes) for each insect species/stage/dose were performed, except for *T. molitor*, where four blocks of five replicates were used. Insect mortality was recorded after 4 days and dead insects were dissected to determine if nematodes were present.

2.4. Analysis

Due to the shape of many of the mortality curves, probit analysis was not appropriate for all treatments. To facilitate statistical comparisons among species and stages, the 10 IJ dose was selected as a threshold level and log-likelihood tests for contingency tables were performed (Zar, 1984). For the log-likelihood tests, treatment mortality was corrected using Abbott's formula (Abbott, 1925).

3. Results

All insects tested were at least marginally susceptible to one or more of the nematode species (Figs. 1–4), and percentage mortality varied depending on the nematode/insect/stage combination. In most cases, mortality increased rapidly to approximately 100%, usually at nematode doses of less than 50 IJs. Most species and stages were highly susceptible to at least one nematode species, except for adult *R. dominica* and *S. oryzae*, for which the highest mortality was 55% (Fig. 4). For *P. interpunctella* and *E. kuehniella*, larvae and adults were highly susceptible, but

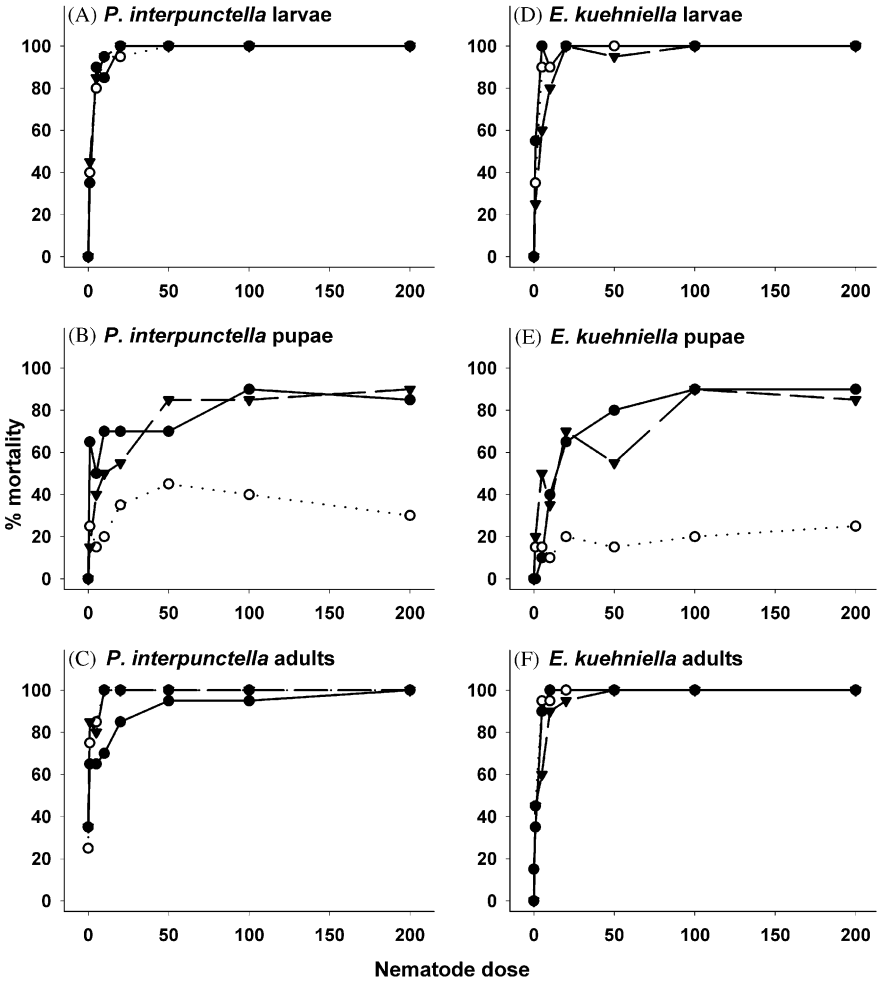

Fig. 1. (A–F) Mortality of larvae, pupae and adults of *Plodia interpunctella* and *Ephestia kuehniella* exposed to 8 IJ doses (0, 1, 5, 10, 20, 50, 100, 200) of three entomopathogenic nematode species, *Steinernema carpocapsae* (filled circle, solid line), *S. feltiae* (empty circle, dotted line), and *S. riobrave* (filled triangle, dashed line).

S. feltiae was not effective against the pupae (Fig. 1). *Tribolium castaneum* larvae were highly susceptible to all nematode species, but pupae were less susceptible to *S. feltiae* and *S. carpocapsae* except at the highest dose and adults were not very susceptible to *S. feltiae* which never caused more than 40% mortality (Fig. 2). All stages of *T. variabile* were highly susceptible to all nematode species (Fig. 3). However, there was considerable variation in susceptibility among the stages of *O. surinamensis* to the three nematode species tested (Fig. 3D–F). *Steinernema riobrave* typically caused higher mortality than the other nematode species across all the stages and insect species.

There were significant differences in the pathogenicity of the three nematode species against the different insects at the 10 IJs dose (Table 1). All three nematode species tested were highly pathogenic to larvae of *P. interpunctella*, *E. kuehniella* and *T. castaneum*, with over 80% host

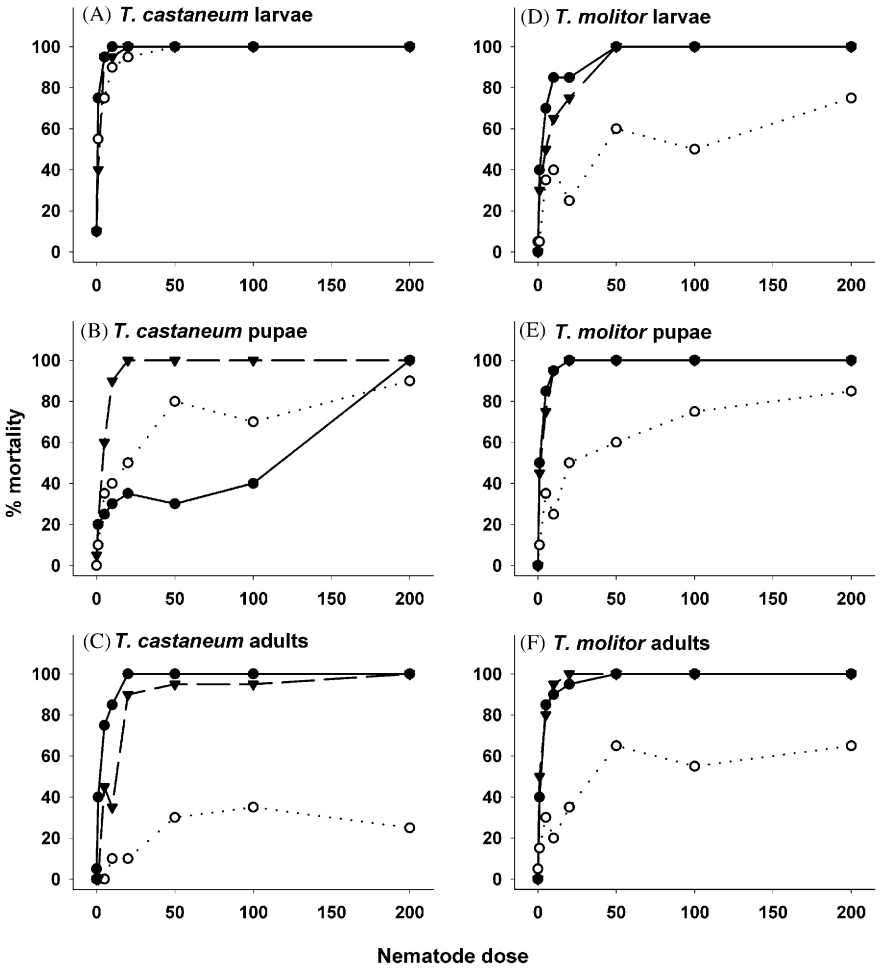

Fig. 2. (A–F) Mortality of larvae, pupae and adults of *Tribolium castaneum* and *Tenebrio molitor* exposed to 8 IJ doses (0, 1, 5, 10, 20, 50, 100, 200) of three entomopathogenic nematode species, *Steinernema carpocapsae* (filled circle, solid line), *S. feltiae* (empty circle, dotted line), and *S. riobrave* (filled triangle, dashed line).

mortality with the 10 IJs dose. *Steinernema carpocapsae* and *S. riobrave* were also highly pathogenic to pupae of *T. molitor* and *T. variabile* and to adults of the two lepidopteran species. *Steinernema riobrave* had the greatest pathogenicity, although in some cases it was the same as one or both of the other nematode species, in all assays, except with *T. castaneum* adults, in which *S. carpocapsae* caused significantly higher mortality (Table 1). *Steinernema carpocapsae* was the next best species overall, causing significantly lower mortality against *P. interpunctella* adults, *T. castaneum* pupae, and larvae and pupae of *O. surinamensis. Steinernema feltiae* only performed well against larvae and adults of moth species and the warehouse beetle, and against larvae of the red flour beetle.

Dissections of the dead insects to determine the presence of nematode development and reproduction provided a measure of host suitability. We used the lowest nematode dose at which

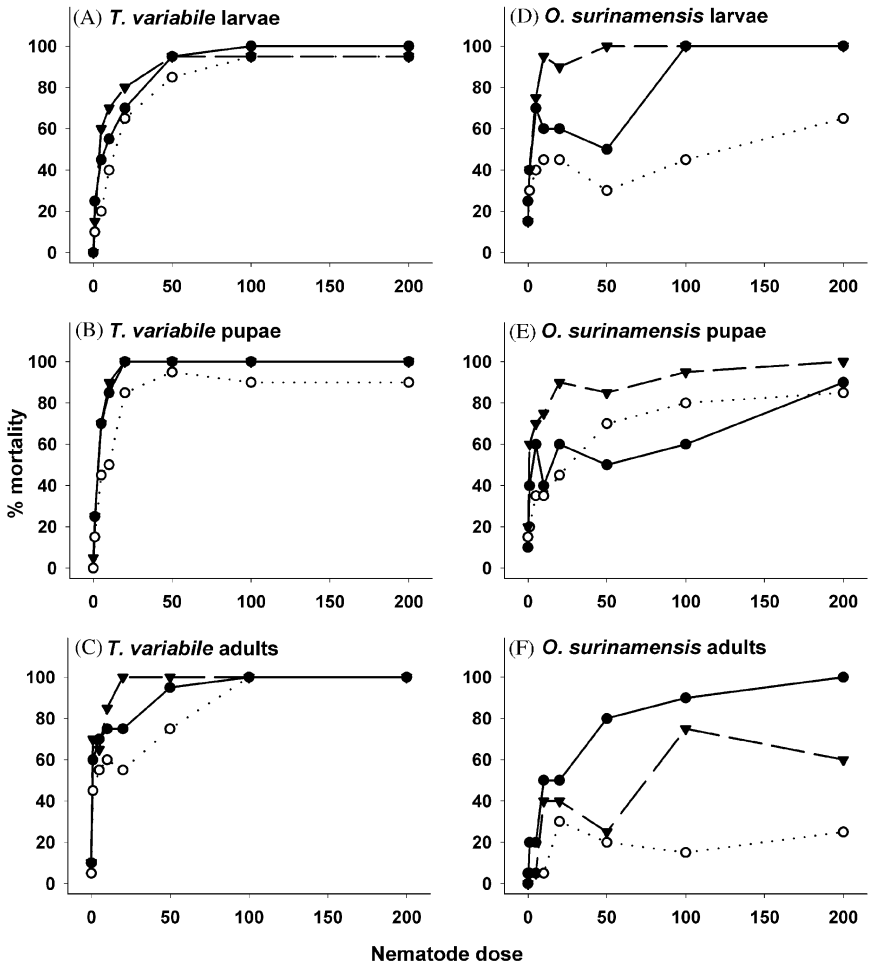

Fig. 3. (A–F) Mortality of larvae, pupae and adults of *Trogoderma variabile* and *Oryzaephilus surinamensis* exposed to 8 IJ doses (0, 1, 5, 10, 20, 50, 100, 200) of three entomopathogenic nematode species, *Steinernema carpocapsae* (filled circle, solid line), *S. feltiae* (empty circle, dotted line), and *S. riobrave* (filled triangle, dashed line).

80% or more of the individuals that died contained nematodes as a threshold value for comparing species and stage suitability. For the tested stages of the two moth species, all the nematode species, except *S. feltiae* against pupae, had a threshold dose of 20 IJs. When pupae were exposed to *S. feltiae*, the maximum percentage of dead pupae with nematodes was only 67%. For *O. surinamensis*, a dose of 100 IJs when exposed to *S. carpocapsae* and a dose of 20 IJs when exposed to *S. riobrave* was needed before 80% of the dead larvae contained nematodes. *Oryzaephilus surinamensis* pupae required 200 IJs for any of the nematode species to reach the threshold and only adults exposed to *S. carpocapsae* reached the threshold (at the 10 IJ dose). For *R. dominica*, the highest percentage with nematodes was only 70%, when exposed to 20 IJs or more of *S. carpocapsae*. For all nematode species tested, *S. oryzae* reached the threshold of 80% when exposed to 50 IJs or less. More than 80% of all stages of *T. castaneum* and *T. variabile*

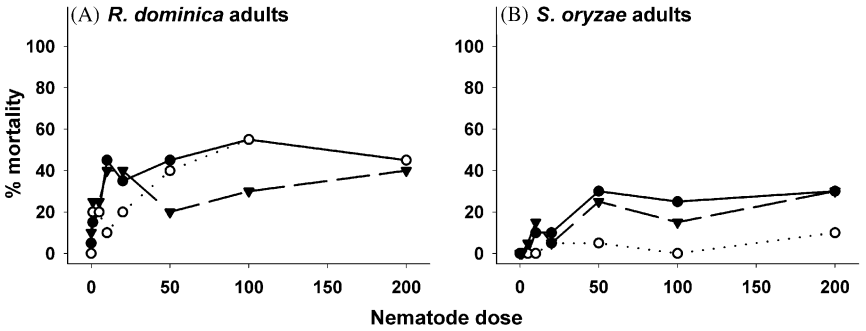

Fig. 4. (A, B) Mortality of adults of *Rhyzopertha dominica* and *Sitophilus oryzae* exposed to 8 IJ doses (0, 1, 5, 10, 20, 50, 100, 200) of three entomopathogenic nematode species, *Steinernema carpocapsae* (filled circle, solid line), *S. feltiae* (empty circle, dotted line), and *S. riobrave* (filled triangle, dashed line).

Table 1
Percentage mortality ($n = 20$) of eight insect species exposed to a dose of 10 IJs of each of three nematode species

Insect species	Stage	Nematode species		
		S. carpocapsae	*S. feltiae*	*S. riobrave*
Plodia interpunctella	Larvae	85 a	95 a	95 a
	Pupae	70 a	20 b	50 a
	Adults	55 b	100 a	100 a
Ephestia kuehniella	Larvae	90 a	90 a	80 a
	Pupae	40 a	10 b	35 ab
	Adults	100 a	95 a	90 a
Tribolium castaneum	Larvae	100 a	90 a	95 a
	Pupae	30 b	40 b	90 a
	Adults	85 a	10 b	35 b
Tenebrio molitor	Larvae	85 a	40 b	65 ab
	Pupae	95 a	25 b	95 a
	Adults	90 a	20 b	95 a
Trogoderma variabile	Larvae	55 a	40 a	70 a
	Pupae	85 a	50 b	90 a
	Adults	75 a	60 a	85 a
Oryzaephilus surinamensis	Larvae	45 b	35 b	95 a
	Pupae	35 b	25 b	70 a
	Adults	50 a	0 b	40 a
Rhyzopertha dominica	Larvae	—	—	—
	Pupae	—	—	—
	Adults	40 a	10 b	35 ab
Sitophilus oryzae	Larvae	—	—	—
	Pupae	—	—	—
	Adults	5 a	0 a	15 a

(—) Stage was not tested.
Different letters in rows illustrate significant differences at the 0.05 level (log-likelihood test for contingency tables).

contained nematodes when the doses exceeded 20 IJs, except for the pupae of *T. castaneum* exposed to *S. carpocapsae* and *S. feltiae*, which never reached this threshold. All the stages of *T. molitor* reached the threshold with a dose of less than 50 IJs for *S. carpocapsae* and *S. riobrave*, but not with *S. feltiae*.

4. Discussion

For many stored-product pests, high mortality was achieved at relatively low nematode doses and *S. riobrave* was either the most pathogenic or of similar pathogenicity as the other nematode species tested. Based on this, *S. riobrave* appears to be a good candidate for further evaluation as a biological control agent for stored-product insects. This species has other attributes that may contribute to its successful use against stored-product insects: it can tolerate warm conditions, being pathogenic at soil temperatures up to 35 °C (Cabanillas et al., 1994), it is an intermediate forager that can seek or ambush its host (Campbell and Gaugler, 1997), and is currently produced commercially. *Steinernema carpocapsae* was the next most efficacious species tested: it was better than *S. riobrave* against adults of *T. castaneum,* but less effective against adult *P. interpunctella*, *T. castaneum* pupae, and larvae and pupae of *O. surinamensis* (Table 1). *Steinernema carpocapsae* is an ambush forager, and this may limit its ability to seek out insects in hidden areas. The low susceptibility of the insects to *S. feltiae*, with only *T. castaneum* adults, and *P. interpunctella* and *E. kuehniella* larvae and adults showing high mortality at a dose of 10 IJs (90%, 95%, and 100%, respectively) could be related to infection preferences.

Most of the insect species and stages tested were relatively susceptible to at least one nematode species, with the exception of adult *S. oryzae* and *R. dominica*. The reason(s) for the lack of susceptibility of these two beetle species is not known. Differences in susceptibility among stages with other species of Curculionidae have been reported, with larval stages being most susceptible (Mannion and Jansson, 1992; Shapiro-Ilan et al., 2002). This could be the case with *S. oryzae*, but because this stage is typically sealed within a kernel, it would not be accessible to the nematodes. The other beetle species tested were relatively susceptible through all the assayed stages. Differences in susceptibility among stages have also been observed in the family Pyralidae, with the pupae being less susceptible than the larvae (Shannag and Capinera, 1995; Shannag et al., 1994). We also found the pupal stage of the tested moths to be less susceptible. Differences in susceptibility can result from a variety of mechanisms. In our bioassay, the insects were confined, so it is unlikely that activity level or avoidance behavior influenced mortality. However, differences in immune response, aggressive behaviors to defend themselves (Drees et al., 1992; Gaugler et al., 1994), and physical barriers to penetration (Gaugler, 1988) may contribute to the differences observed.

Because entomopathogenic nematodes require a moisture film to prevent desiccation and in which to move, they are more typically used in soil environments, and might appear a poor match against stored-product insects. This is certainly true for their use as a bulk grain or processed commodity treatment, but they do have considerable potential as a treatment for hidden refugia and outside spillage or product accumulations. Entomopathogenic nematodes have already been used to control insects in cryptic environments outside the soil. For example, *S. carpocapsae* has been used for controlling cockroaches (Appel et al., 1993) and the codling moth, *Cydia pomonella*

(L.), in fruit bins (Lacey and Chauvin, 1999; Unruh and Lacey, 2001). Chemical insecticides used as surface, empty bin and crack and crevice treatments are typically applied in an aqueous solution (Arthur and Phillips, 2003). If nematodes can be applied in a similar amount of liquid and this provides sufficient moisture for long enough to allow nematodes to find and infect the target, then they could be used as biological insecticides in stored-product situations. Adjuvants have also been added to nematode applications to improve environmental tolerance (Mason et al., 1998) and these could also be tested to extend the window of time to find and infect hosts. Treatment of areas around storage facilities where grain or processed commodity spillage accumulates may be another application area where environmental conditions would be favorable to nematodes. These outside residues may be important sources of insects entering food processing facilities (Campbell and Mullen, unpublished data).

Susceptibility screening under controlled conditions, as reported here, is the first step towards the development of a biological control program. Studies under more natural conditions (rougher substrate, higher temperature, lower r.h.) will be conducted in the next phase to evaluate the range of conditions under which these biological control agents might be utilized and to develop a more accurate prediction of their effectiveness. Based on the characteristics discussed previously, we selected *S. riobrave* for further testing, but *S. riobrave* and *S. carpocapsae* can be considered good candidates for controlling the insect species tested. Using nematodes to control stored-product pests in certain applications may be an alternative that can reduce the dependency on chemicals and add to the tools available to use in integrated pest management programs.

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